THE AMEBACIDAL ACTION OF EMETIN.*

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The much-lauded, and as often denied, efficacy of ipecacuanha in the treatment of amebic dysentery was investigated experimentally by Vedder.[1] Briefly, he found that certain preparations of ipecac exerted a marked lethal action on cultures of saprophytic amebae, paramecia, and balantidia. The efficacy seemed to depend on the emetin content, and this fact pointed to the greater excellence of the Brazil root. While several preparations killed amebae in dilutions of 1:10,000 to 1:50,000, a preparation of de-emetized ipecac failed to kill in 1:5,000 solution. Emetin alone killed the amebae, paramecium, and balantidium in 1:100,000 solution. Vedder also found that when 2 per cent of the fluid extract of ipecac was mixed with agar it exerted a marked inhibitive and germicidal action on *B. typhosus*, *B. paratyphosus*, *B. dysenteriae*, and *St. pyogenes aureus*. This is of importance when one considers the like rôle played by symbiotic bacteria in amebic lesions.

The writer repeated part of Vedder's experiments as in his preliminary note no mention was made of the possible influence of body temperature or the lack of symbiotic bacteria on the results obtained.

TECHNIC.

A 1:10,000 solution of emetin was prepared by adding 0.01 gm. of Merck's emetin to 100 c.c. of sterile double distilled water. The alkaloid was put into solution by adding three drops of $\frac{n}{1}$ HCl. This solution was found to be sterile. Further dilutions were made with aseptic precautions with sterile double distilled water.

The ameba used is apparently of the *limax* type and was isolated on Musgrave and Clegg's medium from tap water in

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[1] *Mil. Surg.*, 1911, 29, p. 318.

Oakland, Cal., in 1909. The culture is composed of the descendants of a single ameba growing along with a small actively motile, non-chromogenic, non-spore-bearing bacillus which produces no distinctive changes in any of the ordinary culture media.

The cultures to be tested were grown for a varying number of days in flasks containing 5 per cent + 1 broth in tap water; autoclaved at 127° C. One c.c. of the ameba culture was pipetted into the bottom of small, sterile, cotton-plugged test-tubes with precaution to avoid contaminating the sides of the tubes, and an equal quantity of any given dilution of emetin was run in on top. After carefully mixing the contents the tubes were incubated at the temperatures and for the length of time detailed below. The contents were then poured into flasks containing 100–200 c.c. of sterile 5 per cent broth, prepared as above, to determine the viability of the amebae. These flasks were then kept at 18°–24° C.

Owing to the rather powerful germicidal action of emetin, control tubes were always made and these were poured into flasks of 5 per cent broth which had been inoculated the day before with a pure culture of the symbiotic bacterium. This culture had been replated several times and numerous examinations proved it to be free of amebae. That this precaution was necessary may be seen from the fact that when the ameba-bacteria mixture was first plated in + 1 agar, amebae wandered out of several well isolated colonies.

EXPERIMENTS.

The action of emetin on the symbiotic bacterium.—1. The bacillus remained alive for several days when a large loop of the densely clouded water of condensation from a + 1 agar culture was inoculated into 1 c.c. of double distilled water. Similar inoculations were made into 1 c.c. of emetin-distilled water dilutions and subcultures into + 1 broth were made immediately afterward and at greater intervals. All negative subcultures were watched for several days to rule out possible inhibition. In all cases there was no immediate germicidal action. The 1:10,000 dilution yielded sterile subcultures when these were made 1.25, 5, and 22.5 hours after inoculation and incubation at 36°–37° C.

2. Emetin-distilled water dilutions 1:10,000, 1:50,000, and 1:100,000 were further diluted with an equal quantity of sterile 5 per cent broth and inoculated and incubated as in the preceding test. Subcultures were made at 48- and 72-hour intervals. Here only the 1:20,000 dilution yielded sterile subcultures.

The action of emetin on the amebae growing with the symbiotic bacterium.—Series 1. The broth culture of amebae was four days old and rich in trophozoits. No cysts were seen in several loopfuls examined. The ameba-bacteria mixtures were not killed by the 1:20,000, 1:100,000, and 1:200,000 dilutions of emetin after one hour's exposure at 37° C. All six flasks examined two weeks later showed many trophozoits and cysts.

Series 2. This was performed as above with a 34-day culture of amebae containing a large number of cysts. Here too the subcultures all showed amebae after one hour's exposure at 39° C. to the same dilutions of emetin.

Series 3. This was performed as above with a 15-day culture of amebae rich in trophozoits. An occasional cyst was found on thorough examination. In this series none of the subculture flasks showed amebae when examined thoroughly on the seventh and eighteenth day after inoculation with the above dilution mixtures, which had been kept at 36°–38° C. for 23.5 hours. Pure cultures of the symbiotic bacterium grew in all six flasks.

Series 4. This was performed with a four-day culture rich in trophozoits and it was only after repeated examinations that a few cysts were found. The same dilution mixtures were allowed to remain in contact for 24 hours at 34°–35° C. Here the subculture flasks were examined on the 10th day and 18th day after inoculation. In the 1:20,000 and 1:100,000 flasks amebae were present in one but absent in the control. Amebae were present in both of the 1:200,000 flasks.

Series 5. A culture of amebae in which no cysts could be found on repeated examinations but rich in trophozoits was used. Emetin 1:20,000 killed the trophozoits in 24 hours, at 37° C. A control, with the sterile distilled water with which the emetin dilutions had been prepared, yielded amebae in the subculture flask.

SUMMARY AND CONCLUSIONS.

Emetin in 1:20,000, 1:100,000, and 1:200,000, dilutions killed the amebae in one of the five series of experiments. (3) after 23.5 hours exposure, at 36°–38° C. None of these dilutions was amebacidal in an hour. It seems fair to presume that when amebacidal action was manifested the emetin acted upon the trophozoits alone and that failure to kill may be attributed to the presence of cysts. While emetin in 1:20,000 dilution was found to kill the symbiotic bacterium in 48 hours it did not exert such an action in 24 hours in the amebae-bacteria mixtures. Exposure to body temperature for 24 hours did not kill this saprophytic ameba.